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The epidermal stratum corneum of the whale

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INTRODUCTION

The anatomy of whale skin was first investigated by Japha (1905), who noted the exceptional thickness of the epidermis. Parry (1949) observed the skin microstructure but was mainly concerned with the elaborate dermal blood vasculature and the blubber. The epidermal histology was examined by Sokolov (1960) in several genera from both the suborders *Odontoceti* and *Mystacoceti*. A general feature was the retention of haematoxylin-stainable nuclei in the most superficial epidermal cells, which, in marked contrast to the stratum corneum of hairy mammals, were not obviously delimited as a separate keratinized layer from the stratum spinosum underneath. From this he concluded that the epidermis in *Cetacea* is either imperfectly cornified or possibly uncornified (Sokolov, 1962).

In most mammals the stratum corneum lacks haematoxylin-stainable nuclear remnants, which are completely broken down during cornification above the stratum granulosum. These epidermal cells with keratohyalin granules are a characteristic feature of terrestrial species. Pyknotic nuclei are retained in the parakeratotic type of stratum corneum which is not formed over a stratum granulosum, and it occurs normally over the moist snouts of some species, and also inside the kangaroo marsupial pouch (Spearman, 1966). Parakeratosis occurs as an abnormal cornification in many epidermal disorders of man and other mammals (Lever, 1967) and as a temporary covering if the normal cornified layer is stripped off with sticky tape (Pinkus, 1951).

Giacometti (1967) examined the epidermal histology of the Fin whale, *Balaenoptera physalus*, in greater detail in several different sites, and did not find a stratum granulosum. The epidermis was keratinized in the perigenital area, but the horny layer was not described. Elsewhere the keratinized cells appeared to have been lost during tissue processing and microtomy. The epidermis was up to 3 mm in depth, much thicker than in any other vertebrate (Spearman, 1966). Long rete ridges projected down from the base of the epidermis into the dermis, a condition first studied in dermopathology and referred to as acanthosis (Lever, 1967); this term can be used equally well for normal animal epidermis with prominent rete ridges, such as the snout epidermis of many mammals including cattle. In the elephant trunk, the rete ridges are much longer than over the body generally (Spearman, 1970*a*). The arrangement may help to anchor the epidermis, although there is no experimental evidence for this.

Hairs do not occur in whales except for one or two aberrant fibres on the head

(Slijper, 1962), and no trace of hair follicles has been found elsewhere in the skin (Sokolov, 1960; Spearman, 1966; Giacometti, 1967), although whales are believed to have evolved from terrestrial hairy mammals. For a fully aquatic animal, a hairy pelt is of no value for thermal insulation once the trapped air is replaced by water, and it may slow down locomotion (Sokolov, 1962). This probably explains the loss of hair in the evolution of *Cetacia*. Spearman (1964) first reported the close association between the presence of hair follicles and eccrine sweat glands and the development in the surrounding epidermis of a stratum granulosum and a stratum corneum without nuclear remnants. This association is clearly shown in rodent scaly tail epidermis, where these granules occur only around the hair follicles, again in embryonic development, where keratohyalin granules first appear in the necks of hair follicles and sweat glands, and also in such disorders as solar keratosis and psoriasis, where sites around the hair follicle are the last to lose the stratum granulosum (Jarrett & Spearman, 1964). In phylogeny, parakeratosis is clearly primitive, since it occurs in the breeding tubercles of certain fish, the earliest instance of epidermal cornification (Wiley & Collette, 1970). Therefore Spearman (1964) suggested that the loss of hair follicles in whales would possibly lead also to loss of a stratum granulosum and a reversion to parakeratosis.

The object of the present investigation was to determine, by histological and histochemical examination of the epidermis, whether normal whale epidermis undergoes parakeratosis.

MATERIALS AND METHODS

A problem in studying whale skin is that specimens for histology are generally obtained many hours after death, and during the interval the skin is exposed to the heat of the sun. As a result, some decomposition inevitably occurs in the epidermis, the upper part of which is frequently lost during tissue processing.

For the present work the Commonwealth Institute of Helminthology supplied satisfactorily preserved skin samples from the Pilot whale, *Globicephala melaena*. The material came from the upper tail fluke and perigenital areas in eight males, four of which were albinos. A less well preserved sample from the posterior dorsal skin of *Balaenoptera physalus* was obtained from the National Jnstitute of Oceanography.

The skin was fixed in 10 % formalin and 7 μm thick vertical paraffin sections were prepared. These were stained in Ehrlich's haematoxylin and aqueous eosin, and by the fluorescent method with Congo red, Titian yellow and Thioflavine t, described by Jarrett, Spearman & Hardy (1959). Under ultraviolet light this technique shows a bright blue fluorescence in parakeratotic stratum corneum, distinct from the brownish red colour of the stratum spinosum underneath.

The following histochemical techniques for protein-bound substances previously found in parakeratotic cells were tested on the paraffin sections:

Protein-bound phospholipids were demonstrated by the blue to black acid haematin reaction as modified by Jarrett, Spearman, Riley & Cane (1965). Protein-bound sulphydryl groups of cysteine were shown by the purple reaction in the dihydroxy dinaphthyl disulphide method of Barrnett & Seligman (1952). Cystine was shown by the peracetic acid oxidation method in which cystine is oxidized pre-

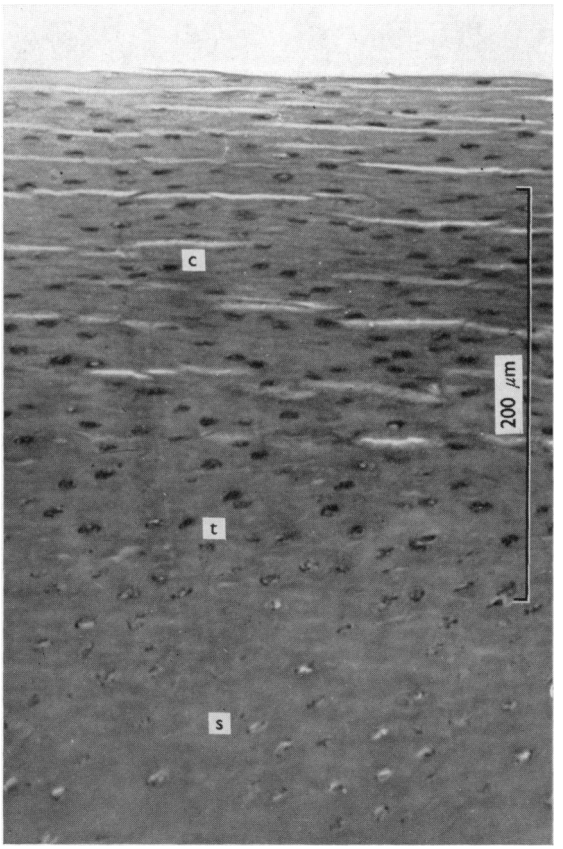

Fig. 1. *G. melaena* dorsal tail fluke epidermis. Haematoxylin and eosin. c, parakeratotic stratum corneum with pyknotic nuclei and tendency to split between flattened cells. t, indistinct transitional zone. s, stratum spinosum.

ferentially to cysteic acid, demonstrable by yellow fluorescence when stained in the basic fluorochrome Thioflavine t (Spearman, 1968; Jarrett & Van Wyk, 1972). Cysteine does not appear to be oxidized, but as a precaution SH groups were blocked in some sections by *N*-ethyl maleimide (Bancroft, 1967) before use of peracetic acid. Nucleic acids, which also fluoresce with Thioflavine t, were removed by digestion in ribonuclease and deoxyribonuclease prior to oxidation in other sections.

RESULTS

In haematoxylin and eosin preparations the epidermal cells gradually became more flattened and the nuclei more shrunken and pyknotic towards the skin surface.There was no stratum granulosum. The superficial cells presumed to be keratinized showed a tendency to flake off during microtomy by splitting along the junctions parallel with the skin surface (Fig. 1). The superficial cells were not so strongly stained with

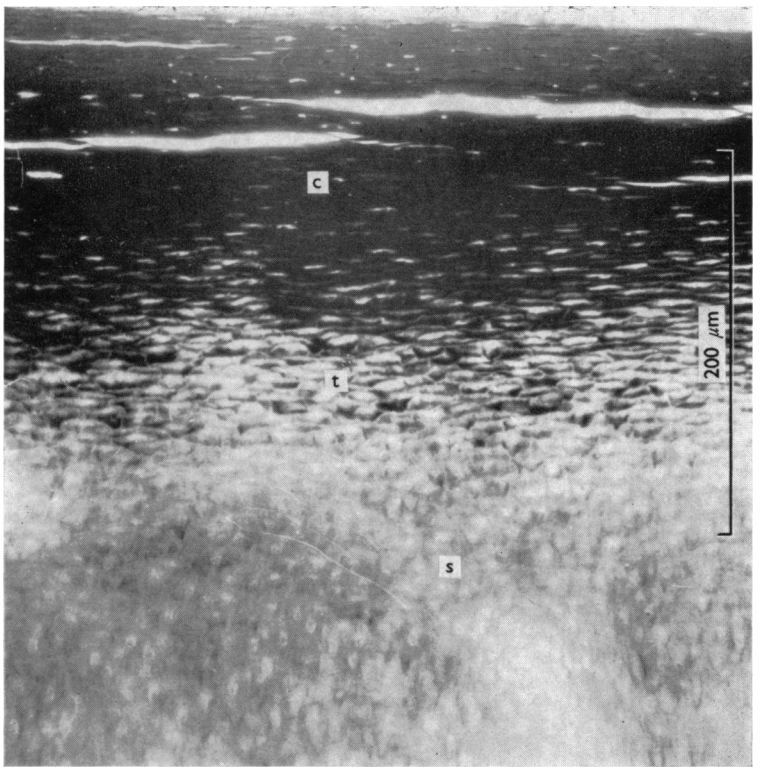

Fig. 2. *G. melaena* dorsal tail fluke epidermis. Acid haematin reaction for protein-bound phospholipids shown by strong blue-black reaction in the statum corneum. c, parakeratotic stratum corneum with tendency to split between flattened cells. t, transitional zone. s, stratum spinosum.

eosin as those in the horny layers of other mammals, including the parakeratotic cells of human skin lesions and the kangaroo pouch.

Delimitation of the stratum corneum was clearly shown by the Congo red method; the superficial layers of cells with the tendency to flake fluoresced a bright blue colour, and the remainder of the epidermis was brown. Cells in the transitional region showed a patchy blue fluorescence. In some specimens, the keratinized layer was lost during processing, but it was demonstrated in some sections in both the dorsal tail fluke and perigenital regions of *G. melaena* and in the dorsum of *B. physalus*.

The acid haematin method for phospholipids showed a pale yellow negative reaction in the stratum spinosum and a strong positive blue black reaction in the superficial epidermis in the same region in which the blue fluorescence occurred with Congo red. In the transitional zone cells there was a patchy reaction for phospholipid (Figs. 2, 3). The strongest concentration of cysteine was also in the superficial part of the epidermis, where a purple reaction occurred, with a deeper coloration in the peripheral region of each cell. The stratum spinosum was stained pink, which is a very weak or negative reaction (Fig. 4).

Fig. 3. *G. melaena.* Acid haematin reaction for phospholipid. Higher magnification of epidermis than in Fig. 2. c, parakeratotic stratum corneum. t, transitional zone.

Cystine was likewise confined to the superficial epidermal cell layer, where there was a pale yellow overall fluorescence in the cells with a deeper yellow coloration at the periphery of each cell (Fig. 5). In contrast, the stratum spinosum had a weak blue autofluorescence after complete removal of nucleic acids by ribonuclease and deoxyribonuclease. In control sections stained with Thioflavine t without oxidation, the stratum spinosum showed some cytoplasmic yellow fluorescence which was removable by ribonuclease. This indication of the presence of RNA was absent from the superficial cell layer.

The stratum corneum demonstrated by these various methods was up to 200 μm in depth, which is extremely thick. The transitional region was some 100 μm in depth. As previously described in whale skin, the base of the epidermis had deep rete ridges projecting into the dermis.

DISCUSSION

The blue cytoplasmic fluorescence demonstrated by the Congo red method in cells with pyknotic nuclei, together with the presence of bound phospholipid, cysteine

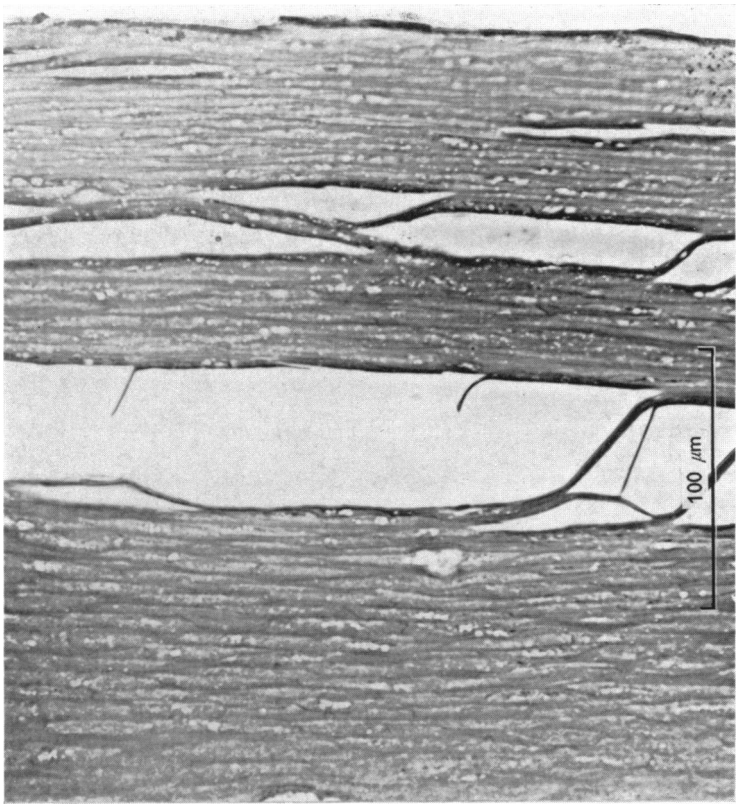

Fig. 4. *G. melaena* dorsal tail fluke stratum corneum. Protein-bound cysteine SH groups by the dihydroxy dinaphthyl disulphide method. There is a purple reaction in the cytoplasm with a stronger reaction at the cell peripheries. Note the tendency to fragment between flattened cells.

and cystine in the superficial region of the whale epidermis, closely parallels previous findings in the parakeratotic horny layer of the human skin disorder, psoriasis, and in the kangaroo pouch epidermis (Jarrett & Spearman, 1964; Spearman, 1966). The chief difference in whale epidermis is the very wide transitional zone, so that in haematoxylin and eosin stained sections there is no clear boundary under the stratum corneum.

The peripheral concentration of cystine in the superficial cells of whale epidermis resembles previous findings on various cornified layers (Jarrett & Spearman, 1964; Spearman, 1970*b*: Fukuyama & Epstein, 1969). Cystine occurs in high concentration in keratin and in cornified cell membranes, but not elsewhere in the epidermis, which is strong evidence of keratinization (Spearman, 1966). This consensus of findings, therefore, supports the contention that whale epidermis undergoes parakeratosis (Spearman, 1964). The finding of cornification in two different sites and in separate genera suggests that whale epidermis is most probably normally keratinized in this way. Previous reports of the absence of a stratum corneum were probably due to its

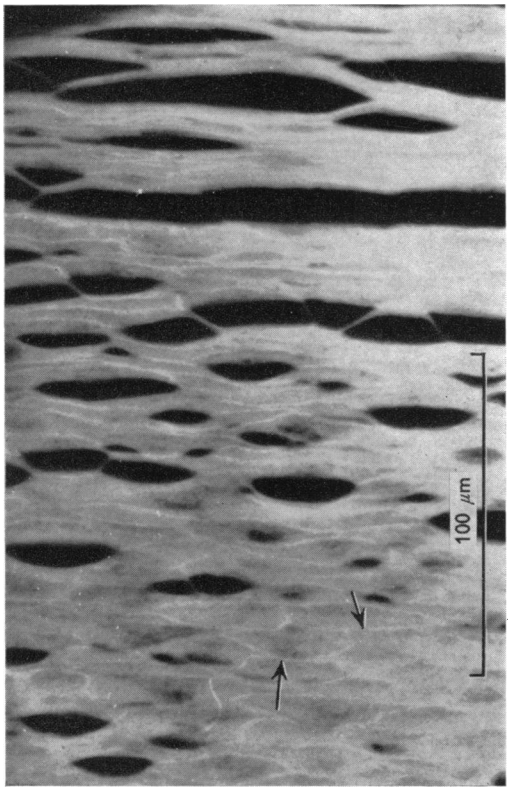

Fig. 5. *G. melaena* dorsal tail fluke stratum corneum. Cystine in keratin by the peracetic acid oxidation method and fluorescence with Thioflavine t. The keratinized cells have a weak yellow cytoplasmic fluorescence and the periphery of each cell has a stronger yellow coloration shown by the arrows. The unkeratinized epidermis does not show this reaction.

loss during tissue processing. Whale epidermis appears to undergo seasonal growth changes, which are shown by variation in thickness of the cornified strata laid down in the outer ear canal (Purves, 1959), and periodic sloughing could possible occur, as in the elephant seal, *Mirounga* (Spearman, 1968). The thickness of the cornified layer may therefore vary from season to season, and this should be noted in future studies. The presence of a broad transitional region in the whale indicates that cornification is a much more gradual process than in parakeratosis in other animals. Indeed, the whale transitional zone is thicker than the entire epidermis of most other mammals.

Previously it was suggested that a cornified layer rich in phospholipid could probably help to waterproof the epidermis, and this would be useful in *Cetacea* (Spearman, 1969, 1970*b*.) Parakeratosis possibly occurs in the normal human vaginal epithelium at one stage (Graham, 1972) and also in the rat vagina (Kahn, 1954). It is also found in mammalian tongue epithelium (Cane & Spearman, 1969), and according to Sicher & Bhaskar (1972) some 75 % of apparently normal human gingivae

are parakeratotic. Clearly, as with acanthosis, what is normal in some situations is abnormal in others, and sometimes, as in the gingivae, distinction is difficult. It is interesting that sites which show normal parakeratosis are either kept moist by glandular secretions or, in aquatic species, by immersion of the body in water.

SUMMARY

The whale epidermis does not develop a stratum granulosum, and the stratum corneum is composed of moderately flattened cells which retain pyknotic nuclei; this represents a form of parakeratosis. The transition between the live stratum spinosum cells and the dead keratinized cells above is much more gradual than in other mammals, even in those with parakeratosis. Apart from this, the presence of protein-bound phospholipid, cysteine and cystine, the absence of RNA, and the retention of pyknotic nuclei in the cornified cells resemble pathological parakeratosis in man and the normal parakeratosis of the pouch epidermis in kangaroos. The presence of cystine, a major constituent of keratin, is the strongest evidence for keratinization in whale epidermis.

It is suggested that parakeratosis in the whale may be a reversion to an evolutionarily primitive form of cornification, associated with the loss of hair follicles in these aquatic mammals. In hairy mammals such a reversion frequently occurs in skin disorders. A thick phospholipid-rich cornified layer probably also helps to waterproof the skin in a fully aquatic animal.

I am grateful to Dr P. A. Riley for the photomicrography of Fig. 5.

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